PROBLEM	POSSIBLE CAUSE	SOLUTION
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.
3. False negative test results	a) Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong sample identification.	Mark the sample I.D. at the time of sample collection.

in vitro diagnostic Reagent, not for medicinal use

J. MITRA & CO. PVT. LTD.
A 180-181, Okhla Indl. Area, Phase-1, New Delhi-110 020, INDIA
Ph: +91-11-47130300, 47130500
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

MN/CSY/115 VER-01 R-04
Rev. Date: Apr.-26

SYPHILIS *i*Clia

Chemiluminescence microparticle immunoassay for Detection of Detection of Antibodies to Treponema pallidum in Human Serum/Plasm

1. INTRODUCTION

Syphilis is a venereal disease caused by infection with the spirochetal bacterium Treponema pallidum(TP). Multiple clinical stages and long periods of latent, asymptomatic infection are characteristic of syphilis. Primary syphilis is defined by the presence of a chancre at the site of incoculation. The antibodies response to the TP bacterium can be detected within 4 to 7 days after the chancre appears. The infection remains detectable until the patient receives adequate treatment. Clinical diagnostic issues realted to syphilis antibodies in human blood by immunoassay. Serological tests (non-treponemal specifc & treponemal specific) are currently the primary method for syphilis diagnosis and management Non-treponemal test (VDRL, RPR, etc.) are generally used for screening and treponemal test (TPHA,FTA-ABS,etc.) are used as confirmatory tests because they detect the presence of antibodies specific to Treponema pallidum

2. INTENDED USE

Syphilis iClia is a chemiluminescence microparticle immunoassay designed for in vitro qualitative detection of antibodies (IgM & IgG) to Treponema pallidum (TP) in human serum or plasma and is used as a screening test for testing of collected blood prior to transfusion. This kit is only operational with J. Mitra CLIA Analyzer.

3. PRINCIPLE

Syphilis iClia is chemiluminescence microparticle immunoassay based on the "Direct Sandwich" principle. The magnetic microparticles are coated with Syphilis antigen with high reactivity for Syphilis. The samples are added in assay cup followed by addition of AE conjugate (Syphilis antigens linked to acridinium ester). A sandwich complex is formed wherein Syphilis antibody (from serum sample) is "trapped" or "sandwiched" between the microparticles coated antigens and antigen labelled with AE conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound AE conjugate is proportional to the concentration of Syphilis antibodies present in the sample. Finally pre-trigger and trigger solution containing hydrogen peroxide and sodium hydroxide solution is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative Light units (RLUs). There is a direct relationship between the amount of Syphilis antibodies present in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the cut-off value.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 50 Tests
- 100 Tests

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer (RA)	Magnetic microparticles coated with Syphilis antigen with preservatives.
Assay Buffer (RB)	Assay Buffer containing BSA with preservatives.
AE Conjugate (RD)	Containing Syphilis antigens linked to acridinium ester with preservatives.
Calibrator-1 (C0)	Cut-off calibrator, BSA in buffer with preservatives.
Calibrator-2 (C1)	Cut-off calibrator, syphilis antibodies in buffer with preservatives.
Control-1 (Q1)	Normal human plasma negative for HIV, HCV, HBsAg & Syphilis with preservatives.
Control-2 (Q2)	Positive for Syphilis antibodies with preservatives.
Reagent Plugs	Silicon caps to cover the opened reagents.

7. STORAGE AND STABILITY

The kit should be stored at 2-8°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8°C.**

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- **Pre-Trigger Solution:** Hydrogen peroxide solution.
- **Trigger Solution:** Sodium hydroxide solution.
- **Wash Buffer:** Phosphate buffered saline solution with surfactant.
- **Assay Cup**
- **J. Mitra CLIA Analyzer**

All materials and analyzer to be used for running the Syphilis iClia shall be from J. Mitra & Co. Pvt. Ltd.

9. SPECIMEN COLLECTION & HANDLING

1. Only human serum or plasma samples should be used for the test.
2. For serum collection use serum vacutainer. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
3. For plasma collection: use Dipotassium EDTA, Tripotassium EDTA, Sodium heparin and lithium heparin gel vacutainer and CPDA blood bag.
4. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
5. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
7. Always use clear specimens. Centrifuge viscus/ thick or turbid specimen at 10,000 RPM for 15 minutes or 4,000 RPM for 30 minutes prior to use to avoid inconsistent result.
8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Syphilis iClia test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. Centrifuge the sample at 10,000 rpm for 15 minutes or 4,000 rpm for 30 minutes.

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
8. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8ºC.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microparticles that may have settled during transport or storage.
5 Once reagents are opened, reagent Plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugs are not used according to the instructions given.
6 To avoid contamination, wear clean gloves when placing a reagent plug on an uncapped reagent bottle.
7. Once a reagent plug has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
8. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8°C (with Reagent Plugs) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent Plug placed) while in refrigerated storage off the system, the reagent kit must be discarded.
9. Run control-1 & control-2 in each assay to evaluate validity of the kit.
10. Distilled or deionised water must be used for wash buffer preparation.
11. Avoid strong light exposure during the assay.
12. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia Analyzer user manual for detailed information on preparing the analyzer.
2. Before loading the Syphilis iClia reagent tray on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microparticles that may have settled during transporation/ storage. Once the microparticles have been loaded, no further mixing is required.

   Important Note: Swirl the microparticle (RA) bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. Once the microspheres have been resuspended, remove the cap and place the reagent plug on the bottle to make it ready to use. Remove the cap of (RA), (RB) and (RD) bottles and place the reagent plugs before use as follow:

   (RA) & (RB) : Natural color plug

   (RD) : Brown color plug
3. Load the Syphilis iClia reagent kit on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 µL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 100 µL.
7. The Syphilis test-specific parameters are stored in reagent barcode placed on the reagent tray and read through barcode reader. In cases, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
8. Run calibration, if required.
9. Mix Syphilis iClia calibrators and controls by gentle inversion before use. Open the the cap and place the Calibrator-1 & Calibrator-2 and control-1 & control-2 vials into each respective sample positions. Read the barcode for calibrator and controls provided with the kit.
10. Press START. The test result for first sample will be obtained at 55 minutes.
11. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculate the results.

Calibration

1. Test Calibrator in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that calibrator and controls values are within the validity range specified in this instruction for use.
2. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless:
3. Recalibrate the analyzer in following conditions:
   a) After each exchange/use of new lot (Test reagent and pre-trigger/ Trigger solution/wash buffer).
   b) Every 15 days and/or at the time of any component to be changed.
   c) Controls are out of validation range.
   d) Required by pertinent regulations.
   e. The kit may also need to be recalibrated after specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the Syphilis iClia.

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

i) Sample to cut-off ratio (S/CO) of control-1 (Q1) must be between 0.001 to 0.5. If it is not so, the run is invalid and must be repeated or calibrated.

ii) Sample to cut-off ratio (S/CO) of control-2 (Q2) must be between 3.0 to 9.0. If it is not so, the run is invalid and must be repeated or calibrated.

iii) Sample to cut-off ratio (S/CO) of calibrator -1 (C0) must be between 0.001 - 0.5. If it is not so, the run is invalid and must be repeated or calibrated.

iv) Sample to cut-off ratio (S/CO) of calibrator-2 (C1)must be between 2.0 to 6.0. If it is not so, the run is invalid and must be repeated or calibrated.

Note: If one of the Calibrator-1 (C0) &/or Calibrator -2 (C1) individual values differ from other 2 replicates ,then analyser automatically disregard that value and calculate the calibrators value with the two remaining calibrator values and provide the result.

RESULT CALCULATION:

The analyzer automatically calculates the sample to cut-off ratio (S/CO) of each sample based on cut-off value using formulas.

a. Cut off value = mean RLU of calibrator-1 + mean RLU of calibrator-2 x Factor (F)

b. Calculation of Sample to cut-off Ratio : Sample cut-off Ratio is calculated as follows:

Sample cut-off Ratio (S/CO) = RLU of Sample / Cut-off value.

Note: Factor (F) is batch specific and is provided in the calibrator barcode.

14. INTERPRETATION OF RESULTS

a. If the Syphilis S/CO is < 1.0 then interpret the sample as Negative for Syphilis antibodies.

b. If the Syphilis S/CO is > 1.0 then interpret the sample as Positive for Syphilis antibodies.

15. PERFORMANCE CHARACTERISTICS

A) Diagnostic Sensitivity and Specificity: The Performance of the Syphilis iClia with reference to sensitivity and specificity was evaluated in-house with the panel of 86 negative and 20 Syphilis positive samples. The performance is also checked with fresh clinical negative (200) and Syphilis Positive (30) samples. The results of all the positive and negative samples were compared with commercially available licensed test kit. The results of the in-house study done are as follows:

No. of Samples	Status	Syphilis iClia		Commercially available Syphilis ELISA	
		Positive	Negative	Positive	Negative
50	Syphilis Positive	50	0	50	0
286	Syphilis Negative	0	286	0	286

Sensitivity : 100% **Specificity :** 100%

B) Analytical Specificity :

The analytical specificity of the Syphilis iClia Test kit is checked to check the potential for false results with 10 cross-reacting specimen; HIV, HCV, RA, CRP and antenatal. The specificity on all above samples tested is 100%. The analytical specificity of the test kit is also checked with potentially interfering substances /samples to check the potential for false results arising from interference from potentially interfering substance .There was no interference with the test results when biomolecules; Bilirubin (20 mg/dl), Hemoglobin (500 mg/dl),Triglyceride (1000 mg/dl), Total protein (10 g/dl), RF (1000 ng/ml), ANA (400 mg/ml) & HAMA positive human plasma (600 ng/mL) were added to the test specimen with much higher level in normal human blood.

C) External Evaluation:

The performance of 3 lots of Syphilis iClia with reference to sensitivity and specificity is evaluated by National Institute of Biologicals. Following are the results:

Sensitivity: 100% **Specificity:** 100%

Precision: Precision is checked by running Syphilis iClia test in 10 replicates (Intra assay variation, Inter assay variation) and Inter Machine variation with Kit controls(Contro 1& Control 2) , 2 Syphilis positive samples; one strong positive and one weak positive .The CV% in Sample RLU to Cutoff ratio (S/CO) of both the controls and positive samples is within 10% for intra-assay variation and within 15% for inter-assay and inter-machine variation.

16. LIMITATION OF THE TEST

1. Syphilis iClia designed for testing syphilis antibodies in human serum and plasma. Other body fluids and pooled samples are not recommended in this assay.
2. Any result derived from the test of pooled serum/plasma may not be interpreted correctly based on the current criteria.
3. Syphi iClia testing alone cannot be used to diagnose syphilis even if antibodies against syphilis are present in human serum or plasma.
4. A negative test result at any time does not preclude the possibility of exposure to, or infection with syphilis.
5. **This is only a screening test.** All samples detected reactive must be confirmed by using other EIA assays/ confirmatory assays.
6. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data should be considered. The results should be reported only after complying with the above procedure.

17. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

18. REFERENCES

1. Consistency Between Treponema pallidum Particle Agglutination Assay and Architect Chemiluminescent Microparticle Immunoassay and Characterization of Inconsistent Samples. Zhiyan L, Meiling W, Ping L, Jinhua D, Zhenlin Y, Zhenru F.J Clin Lab Anal. 2015 Jul;29(4):281-4. doi: 10.1002/jcla.21765. Epub 2014 May 19.PMID: 24840601
2. The Laboratory Diagnosis of Syphilis.Satyaputra F, Hendry S, Braddick M, Sivabalan P, Norton R.J Clin Microbiol. 2021 Sep 20;59(10):e0010021. doi: 10.1128/JCM.00100-21. Epub 2021 May 12.PMID: 33980644
3. Evaluation of a new chemiluminescence immunoassay for diagnosis of syphilis; Xiaohui Mo,1 Yuelan Jin,1 Yang Yang,1 Weizhong Hu,1 and Weiming Gu1
4. Antonella M, Vittorio SA, Francesca C. Evaluation of LIAISON Treponema Screen, a Novel Recombinant Antigen-Based Chemmiluminesnce Immunoassay for Laboratory Diagnosis of Syphilis. Clin Diagn Lab Immunol. 2005;12:1231–1234.

19. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls/ Calibrator deterioration due to improper storage or used after expiry.	Ensure calibration is done after 15 days and use controls/ Calibrator within 30 days once opened and check storage temp. It should be 2-8ºC.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8ºC.
	d) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
2. False Positive test results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer test specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.